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Microbial and physico-chemical analysis of composting process of wheat straw

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Composting is a method of solid waste management to convert organic matter into agriculturally useful humus like substance to enhance nutritional and physical quality of soil. In an attempt to produce quality compost, studies were conducted for six months to investigate the physiological and biochemical changes during the composting of wheat straw. To monitor the composting process a consortium of bacterial isolates (*Bacillus subtilis* D3L/1, *B. subtilis* B1U/1 and *Pseudomonas* sp. RAT/5) (10^7 CFU/mL) was used. After 60 d of incubation, the wheat straw turned black, showed distinct physical changes and emitted odour. Periodical analysis of C/N ratio revealed that ratio of final product was 17:1. Other parameters like NO_3^- (1.5%) and NH_4^+ ion (0.3%) concentration along with *p*H (7.0±0.2), K^+ (44%) and PO_4^{-3} (15.46-33.18 mg/Kg) concentration were also recorded. Maximum activity of amylase together with protease and cellulase were recorded after 60 d of incubation and found to be 44.9-54, 62.28-193.02 and 29.1 U/g, respectively; whereas dehydrogenase activity reached its maximum (4168 U/g) at 30th d. *Salmonella* population did not appear throughout the study period. The load of fastidious organisms was 5.3-5.7 U $\log_{10}$ CFU/g up to 30 d that disappeared thereafter, but the coliform population was predominating. Certain fungal strains were detected with potato dextrose agar medium. Physiological, biochemical and morphological character of the final product revealed that aerated static pilling is suitable for compositing of wheat straw.

Keywords: C/N ratio, composting, enzyme, nonpathogenic, static pile

Introduction:

Composting is a process of exothermic biological oxidation of various organic wastes in the presence of air and involving microorganism. Through microbial decomposition, the organic matter is stabilized, matured and deodorized into a product rich in humic substances that can be used as organic soil conditioner, easy to store and distribute[1-3]. An adequate supply of nitrogen (N), phosphorus (P), potassium (K) and other fundamental nutrients are essential to sustain crop productivity. Farmers often utilized the composting procedure to add stabilized organic matter into soil for improving the soil fertility and crop productivity.

Composted manure has been recognized as an effective way to partially solve the growing concern of solid waste management[4] by reducing the volume and weight of the organic waste, as well as controlling the soil pathogens[5-8]. However, immature composts may contain growth inhibiting substances like salts, free ammonia, phenolic substances, heavy metals and organic acids. Although several reports are available concerning the chemical composition and dynamics of the microflora during the composting[9-13], but little is known about the microbial diversity throughout the decomposition of organic fractions of household wastes. As microorganisms play key role in the process, monitoring of microbial succession is important for the effective management of the composting process. The appearance of certain microorganisms reflects the quality of mature compost[14,15].

Evaluation of the composting process for stability (maturity) and quality is based on physical as well as chemical parameters of the final product that depends on the metabolic activity of microorganisms involved in the decomposition[16-19]. The present communication deals with the identification of essential parameters for composting of wheat straw.

Material and Methods

Experimental Design

Three sets of piles (C1, C2 and C3) of suitable size were made with wheat straw (7 kg each) in plastic containers. In set C1, the substrate was simply dumped; in set C2, perforated pipe was dipped up to base of the substrate; and set C3 had larger surface area with manual mixing of the composting substrate.

Microorganisms Used

Bacterial isolates, *viz.*, *Bacillus subtilis* B1U/1, *B. subtilis* D3L/1 and *Pseudomonas* sp. RAT/5[20],

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were grown using fermenter and a mixture (1:1:1) of these isolates were inoculated (10^7 CFU/mL each) into the substrate for the production of compost. Further, the moisture content of the substrate was maintained to 65%.

Media Used

To check the load of nonpathogenic organisms, viable counts were made on nutrient agar (NA) [(g L^{-1}): peptone 5.0, beef extract 3.0, NaCl 5.0, agar 15.0]; potato dextrose agar (PDA) [(g L^{-1}): peeled potato 200.0, dextrose 20.0, agar 15.0]; starch agar (SA) (nutrient agar supplemented with 1% starch); and Czapek mineral salt agar (CMSA) [(g L^{-1}): $NaNO_3$ 2.0, K_2HPO_4 1.0, $MgSO_4$, $7H_2O$ 0.5, carboxy methyl cellulose 5.0, peptone 2.0, agar 20.0, with *p*H 7.0±0.2].

For cultivation of pathogenic organism, blood agar [(g L^{-1}): proteose peptone 15.0, liver extract 2.5, yeast extract 5.0, NaCl 5.0, agar 15.0, sterile defibrinated blood 7% (v/v)]; shigella-salmonella agar [(g L^{-1}): peptic digest of animal tissue 5.0, beef extract 5.0, lactose 10.0, bile salt mixture 8.50, sodium citrate 10.0, sodium thiosulphate 8.50, ferric citrate 1.0, brilliant green 0.00033, neutral red 0.025, agar 15.0]; and eosine methylene blue agar [(g L^{-1}): peptone 10.0, lactose 5.0, sucrose 5.0, dipotassium phosphate 2.0, agar 13.5, Eosine Y 0.4, methylene blue 0.065] were used.

Sample Analysis

Sub-samples (5 g each) and in triplicate were taken from three different layers (upper, middle and lower) at 15 d interval from d 1 of composting process[21]. The first sub-sample was stored at 4°C to provide a sample library; the second sub-sample was used for the physicochemical analysis; and the third sub-sample was used for the microbiological analysis. For microbial analysis, the inoculated culture plates were incubated at 37°C for 48 h.

Physico-chemical Properties

At each sampling period, physical characteristics including colour by visual observation, moisture content by gravimetric analysis (by deduction of water loss), odour through olfactory judgement, texture sensing coarseness, electrical conductivity (aqueous 1:5 w/v) using an electrical conductivity probe and *p*H (1:10, aqueous extract) were determined. The chemical composition, such as, organic carbon[22], nitrogen[23], phosphate[24] and potassium[25] concentrations, of the sub-samples was determined.

Enzyme Assay

Amylase[26], cellulase[26], protease[27] and dehydrogenase[28] activity of each sample was determined. For amylase and cellulase, an enzyme unit is defined as the amount of 1 m*M* of glucose released from the substrate in 1 min at 90°C and expressed in U/g against glucose standard. For protease one unit is defined as the amount of enzyme hydrolyzing casein to develop colour equivalent to 1.0 *M* of tyrosine per min in standard assay condition and expressed as U/g. The dehydrogenase activity is expressed[29] as triphenylformazan formed (g/g).

Statistical Analysis

All experiments were conducted in triplicates. The values are mean±SD[30].

Results and Discussion

Evaluation of Physico-chemical Properties

During the entire process, a gradual change of quality of the raw material was monitored. It was observed that, after 60 d, it got stabilized and appeared black humus-like substance in set C2, but set C1 and C3 required an extended period of 75 d for the same.

Odour

Odour of composting feedstock is associated with the release of carbon in gaseous form, volatile organic acids (VOAs) or other chemical compounds[31-33]. The organic substrates are of high odour potential and often accumulate in excess (becoming phytotoxic) under air-limited and/or low *p*H conditions[34,35]. In set C1, ammoniacal odour was most profound up to 45 d. In set C2, ammoniacal odour was not recorded at all, which might be due to the better aeration that sped up the process of decomposition. In set C3 (without air pipes), still no odour was generated and that too probably because of periodical mixing, which improved the oxygen supply in the pile and provided better activity of consortia. After complete decomposition, black coloured humus like substance was generated in each set having an earthy smell.

Electrical Conductivity

Electrical conductivity (EC), an indirect measurement of the soluble salts of a sample, is used as chemical indicator of the composting status. EC values increased gradually as the process continued in all the 3 sets and reached to a stable value at the end

phase of decomposition, starting from 120 d in set C2 (Fig. 1A). No major variations were observed in all the 3 sets because of, in all possibility, the use of common raw material. The EC values from start of the decomposition of raw material to the end ranged between 0.6-3.85 mS/cm. Forced aeration has been found to enhance the rate of decomposition of composting of poultry litter with a final conductivity of 3.6 dS/m[19]. However, composting of sludge sample showed a much higher value (60 mS/m) of conductivity[28].

C/N Ratio

High C/N ratio indicates the presence of unutilized complex nitrogen[28,36], whereas completion of the process (compost maturity) is indicated by the reduction of ratio to 25:1 or 30:1[37]. In the present study, high C/N ratio (128:1) was noticed at the initial stage, which decreased gradually with the passage of substrate decomposition (Fig. 1B). The results corroborated with the study of Hadas and Portnoy[38]. In set C2, within 75 d of decomposition, the C/N ratio decreased to 27:1 and remained stable up to 150 d,

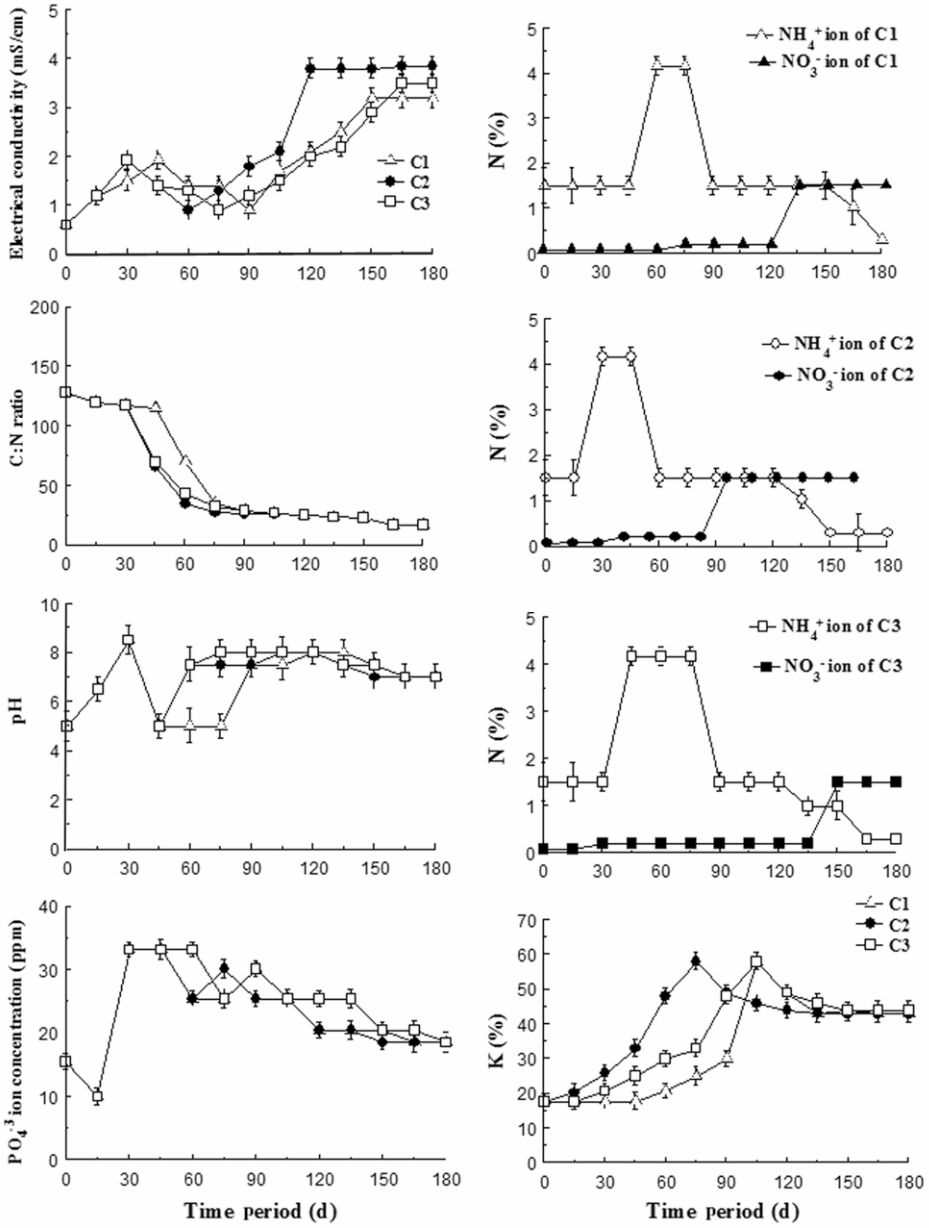

Fig. 1—Physico-chemical changes during the decomposition of wheat straw in different composting piles. Results are the mean±SD value from three independent experiments. [C1: Piling; C2: Aerated static pile; C3: Piling with larger surface area.]

which further decreased to 17:1 in the final product. A similar trend was observed in other sets, but the C/N ratios recorded after 75 d of decomposition were 35:1 and 32:1, respectively for sets C1 and C3 (Fig. 1B). The final C/N ratio of 17:1 indicates the high rate of decomposition in all the sets. The decrease of C/N ratio was the result of transformation of organic carbon into carbon dioxide, followed by a reduction in the organic acid content[33,39].

pH

In the experimental sets, initial *p*H value of substrate was found to be 5.0. As the decomposition proceeded, the *p*H value gradually increased up to 8.0, while the final value was recorded as 7.0 (Fig. 1C), indicating the stability of organic matter. In set C1, the acidic *p*H changed to alkaline after 75 d of decomposition; while the same point was reached within 60 and 45 d in sets C3 and C2, respectively.

Earlier studies have indicated that *p*H range of 5.5-9.0 was suitable for microbial decomposition of organic materials, while the composting process was most effective at *p*H values between 6.5 and 8.0[20,40]. Beyond such range of *p*H, microbial activity was compromised and even inhibited. The increase in *p*H is generally thought to be the result of volatilization and microbial decomposition of the organic acids and subsequent release of ammonia through mineralization of organic nitrogen sources[9]. Also, the alkaline *p*H is preferred for controlling of pathogenic fungi[36].

Ionic Condition

In the present study, variation in phosphate ion concentration during composting was observed between 15-33 mg/Kg (Fig. 1D). In set C2, the ion concentration reached its maximum at 45th d, followed by a sharp decrease and finally a steady state after 75 d. A similar trend was observed in other sets but steady state appeared much later in set C1 (105 d) and set C2 (90 d).

The results show that NH_4^+ ion concentration reached to its maximum at 60th d of decomposition in set C1 (4.1%), which came below 0.3% at the end of composting (Fig. 1E). A similar trend was also observed for the other sets, but the maximum concentration for C2 (4.17%) and C3 (4.2%) was observed at 30th and 45th d of decomposition, respectively. In case of NO_3^- concentration during composting, the concentration in set C2 initially increased slowly and reached to 1.51% after 90 d of decomposition. Thereafter, the concentration got stabilized. Sets C1 and C3 also showed a similar pattern but the time periods for concentration stabilization for them were 120 and 135 d, respectively (Fig. 1E). Thus at the end of decomposition, in all the sets, mature compost showed 1.51% of NO_3^-. In other such studies, NH_4^+ concentration in different stages of composting ranged from 0.2 to 4.2%, while the NO_3^- concentration ranged from 0.1 to 0.5%[33,36]. The decrease of NH_4^+ and increase of NO_3^- ion concentration was largely due to microbial nitrification and transformation of NH_4^+ to NO_3^- [19,34]. The relative proportions of the nitrogenous species (NH_4^+ & NO_3^-) indicated the stabilization of the process and conversion of ammonia to nitric acid[36].

Initially, the K^+ ion concentration was relatively high (17%), which steeply reached to 58% with the advancement of decomposition and stabilized at 44% at the end of composting. All the 3 sets showed a similar pattern but had varied time periods to reach the peak concentration, ***i.e.***, C2-15 d, C3-30 d and C1-45 d (Fig. 1F).

Enzyme Activities

The enzymatic activities in compost piles are effective indicators for stress or adaptive practices of the microorganism to different environmental conditions, particularly to feed stock sources. Various hydrolytic enzymes can control the rate of decomposition of complex polymers during composting[41]. To find out the combined effect of cellulase, amylase, protease and dehydrogenase on substrate by the consortium of isolates, the total biological activity of aqueous extract of compost sample was measured (Fig. 2). Since the dehydrogenase is an intracellular enzyme, samples were treated 24 h before its quantification.

Cellulase activity reached its maximum at 60th d of decomposition in set C1 (29.1 U/g) and set C3 (22.1 U/g). However, in case of set C2, though the amount of enzyme released was lower (16.44 U/g) but the activity reached its maximum at 30th d of decomposition, and stabilized after 90 d. On the other hand, cellulose activity was stabilized in sets C1 and C3 after 135 and 105 d, respectively.

The maximal amylase activity ranged from 44.9-54 U/g and it was obtained in 60 d for set C1 and C3, while set C2 showed the highest activity in 30 d. Further, the enzyme activity was stabilized after 120,

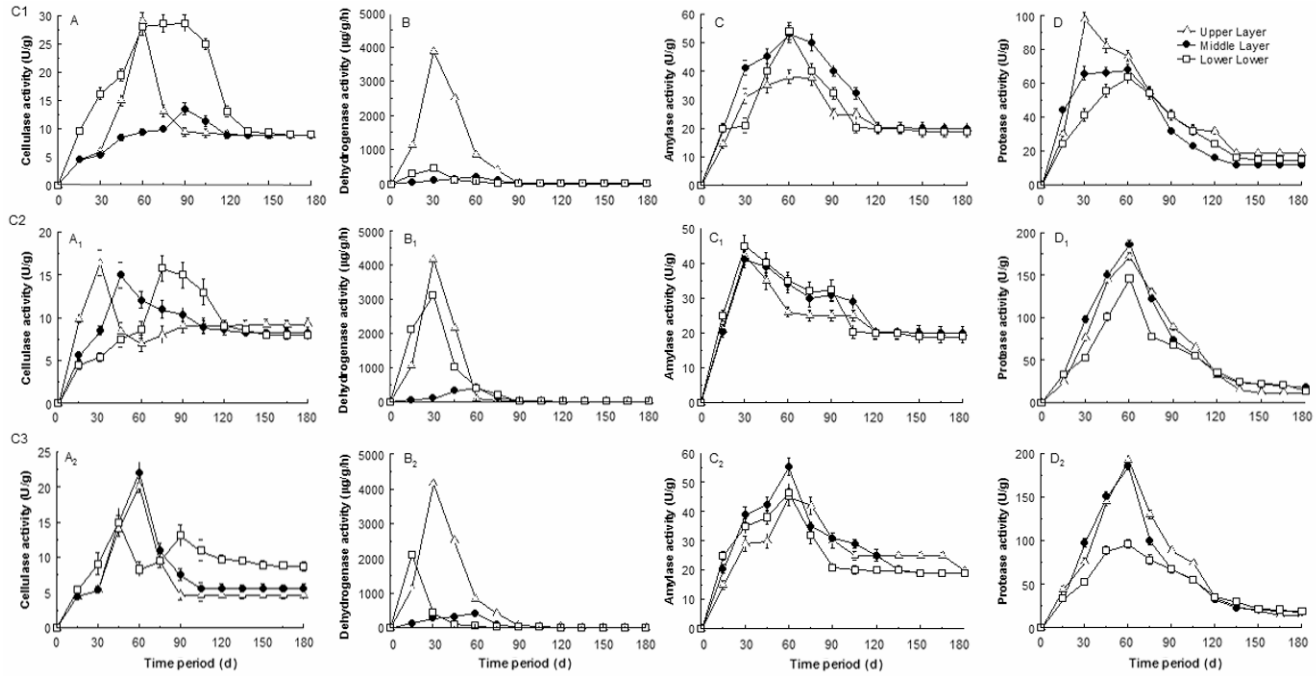

Fig. 2—Changes in enzyme activity during composting. Results are the mean±SD value from three independent experiments. [A, A_1, A_2: Cellulase activity; B, B_1, B_2: Dehydrogenase activity; C, C_1, C_2: Amylase activity; D, D_1, D_2: Protease activity by the combined action of *B. subtilis* B1U/1, *B.subtilis* D3L/1 and *Pseudomonas* sp. RAT/5; C1: Piling; C2: Aerated static pile; C3: Piling with larger surface area]

105 and 135 d of composting in set C1, C2 and C3, respectively.

The maximum activity (62.28-193.02 U/g) of protease was recorded at 60th d of decomposition in sets C2 and C3, while it was recorded at 30th d in set C1. Later, the activity of protease was found stabilized in sets C2 and C3 after 150 d, and in set C1 in 135 d.

Dehydrogenase activity (DHA), an indicator of the microbial action in soil, showed a net increase between 296-4168.1 g/g/h within 45 d of decomposition. The activity reached its maximum (C1, 3881 g/g/h; C2, 4168.1 g/g/h; C3, 4100 g/g/h) at 30th d of decomposition in each set, followed by a gradual decrease up to 90 d. Not only that, these values of dehydrogenase activity varied in the lower layer of each pile. A net decrease of the DHA was recognised at the maturation stage[35,42].

Studies have revealed that compost piles are excellent source of cellulolytic decomposers[43]. Further, Tiquia and Tam[21] have reported that O_2 transformation is necessary for the growth of aerobic organism. In set C2, air circulation facilitated the growth and colonization of organisms; hence, enzymes production for efficient decomposition reached its maximum within 30 d and the production was stabilized after 105 d. However, rest two sets required maximum of 60 d to reach the optimum of enzymes production and, within 135 d of decomposition, the activities were found to be stable. The amount of enzyme released was found significantly high in set C2 as compared to sets C3 and C1,. Also in set C3, manual mixing possibly made the aeration of the substrate that supported better growth and enzyme production than set C1, where substrates remained undisturbed (less aeration). The studies of Raut *et al*[44] and Castaldi *et al*[45] regarding production of hydrolytic enzyme in such situations also supported the present results.

Biological Parameters for Composting

Total Viable-Cultivable Nonpathogenic Bacteria

The periodical changes in microbial diversity of the composting piles are presented in Fig. 3. In viable counts, it was observed that layers of the piles were dominated by *B. subtilis* B1U/1, *B. subtilis* D3L/1 and *Pseudomonas* sp. RAT/5[20], using the media like NA, SA, CMSA. After 24 h of incubation, the count was 40-400 CFU/plate. For detection of fungal diversity, PDA plate was used, which showed negligible count of CFU that declined further as the decomposition proceeded. Microbial diversity was

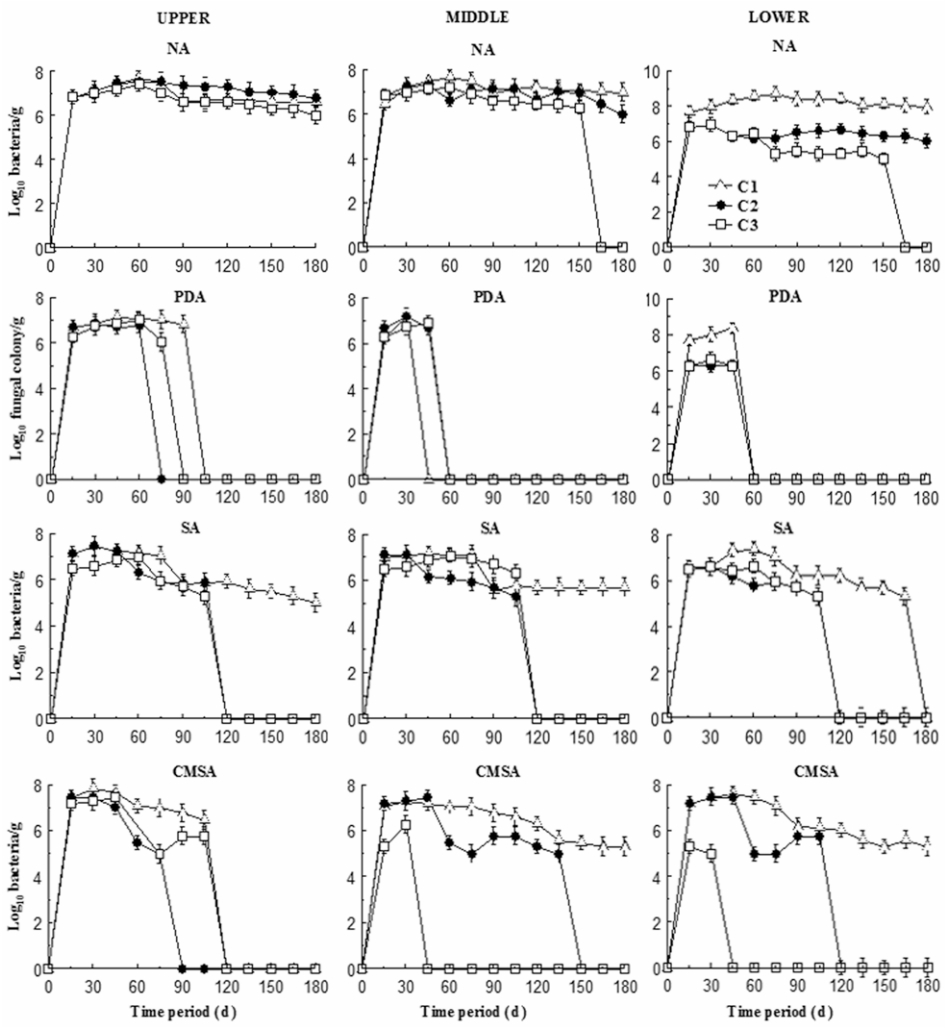

Fig. 3—Periodical analysis of microbial population in different layers (upper, middle & lower) of composting material. Results are the mean±SD value from three independent experiments. [NA: Nutrient agar; PDA: Potato dextrose agar; SA: Starch agar; CMSA: Czapek mineral salt agar; C1: Piling; C2: Aerated static pile; C3: Piling with larger surface area]

variable (5.3-8.7 U, *i.e.*, $\log_{10}$ CFU/g) up to 90 d and stability appeared there after. Microbial diversity reflects the quality of matured compost and dominance of a particular species indicates the increased number of cell generation[14,15] resulted from better metabolic activity supported by availability of oxygen, which was inversely supported in case of set C1 (static pile).

Total Viable-Cultivable Pathogenic Bacteria

Additional benefits of composting has been to control pathogenic microorganisms that to avoid a contamination of waste[46]. The presence of pathogen in compost was investigated by targeting the load of coliforms, fecal coliforms, *Escherichia coli*, *Shigella*, *Salmonella* and fastidious pathogens having haemolytic activities during the entire process[28]. Coliform bacteria were recorded in upper and middle layer of set C1 up to 90 d, whereas no colony was found in lower layer. In case of set C2, coliforms were found till 135 d of decomposition but the number of colonies gradually decreased and finally none of the coliforms were detected in matured compost. In set C3, coliforms were detected only up to 30 d in each layer (Fig. 4). Absence of *Shigella* and *Salmonella* in the entire decomposition of all the piles was corroborated. Martens[47] reported that these bacteria were destroyed when the temperature reached 55°C. *Salmonella* species are regarded as the problem of hygienic quality of the compost. This is probably related to the fact that these bacteria are ubiquitous and of rapid growth. During the early stages of decomposition, fastidious organisms were recorded in the blood agar plates up to

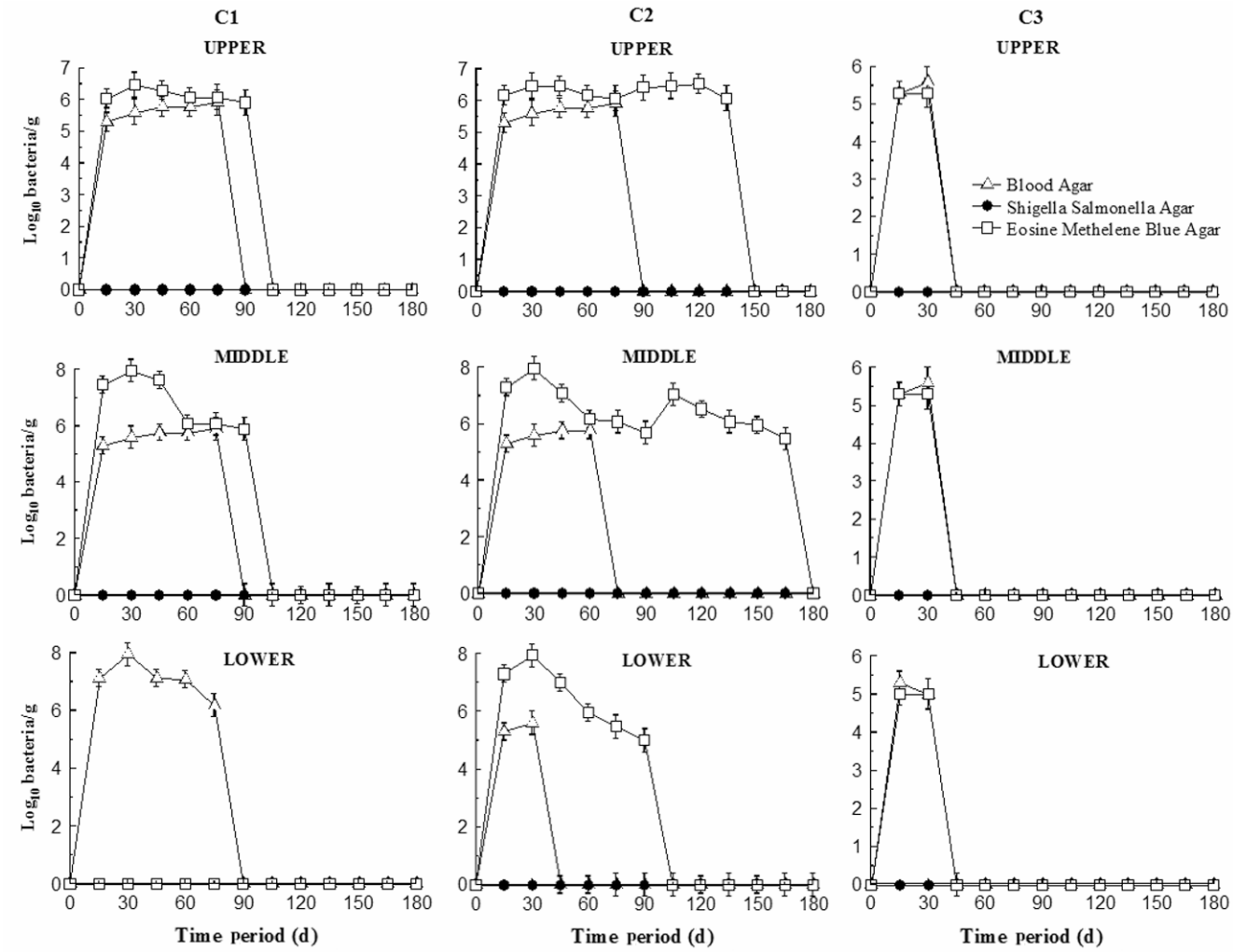

Fig. 4—Viable count of pathogenic organism during decomposition of wheat straw. Results are the mean±SD value from three independent experiments. [C1: Piling; C2: Aerated static pile; C3: Piling with larger surface area; Upper: Upper layer of pile; Middle: Middle layer of pile; Lower: Lower layer of pile]

30 d (5.3-5.7 U $\log_{10}$ CFU/g) in set C2 and C3, with an exception of set C1 where these organisms were detected till 75 d of decomposition but no haemolytic activity was observed.

Conclusion

Static pile is the simplest way of composting with least operational cost and less investment. The piling system can be operated particularly where land is limited and/or even close to habitation. The degradable organic matter decomposes slowly due to passive movement of air through the pile but the scope of aeration facilitates the decomposition. Among the techniques attempted in static pile, the set C1 (dumping of substrate) was found less effective, since the in flow or the out flow of gasses were only through passive diffusion. Decomposing efficiency in set C3 was better than set C1 but less than set C2, where technique involve helped to exchange gases. Thus, the present study recommends for the use of consortium of microbial isolates in static pile with simple aeration device that can decompose organic matter efficiently in terms of less time requirement and almost no additional energy to make the process cost effective.

In view of such unique features, the option of using consortium of indigenous natural isolates for composting is far more appropriate under the Indian context where urban landscapes are changing rapidly and where communities (even in peripheral settlements) are increasingly expect improved quality of life, least of all, uncontaminated water, odour free air and residue free crop products.

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